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# Habitual behaviour associated with exposure to high-calorie diet is prevented by an orexin-receptor-1 antagonist

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## Abstract

Habitual actions, which are associated with addictive behaviours, contribute to the loss of control of food seeking seen following exposure to calorie-dense foods in rats. Antagonism of orexin-receptor-1 (ORX-R1) has been shown to reduce a range of stimulus-driven feeding behaviours, but have yet to be implicated in the regulation of habitual actions. In the current study, male Long-Evans rats were given 'binge-like' access to high-calorie diet (HCD) or standard chow diet, and were subsequently trained to press a lever for food outcome. When lever responses were tested following outcome devaluation, chow-fed rats displayed goal-directed actions, whereas HCD-exposed rats displayed habitual actions. In study 1, it was shown that systemic administration of the ORX-R1 antagonist, SB-334867, prior to test restored goal-directed behaviour in HCD-exposed rats. In study 2, intra-nigral administration of SB-334867 similarly restored goal-directed behaviour, thereby implicating the substantia nigra as an important site for this effect. This study demonstrates that targeting ORX-R1 reduces habitual food seeking in male rats which may be important for understanding and treating compulsive feeding, obesity and binge eating disorder. This study also implicates the lateral hypothalamus, where ORX is produced, in mediating the expression of habits for the first time, and thus extends on the neurocircuits known to regulate habitual actions. Further investigation is required to determine whether the same effects are also seen in female rats, given that there are recognised sexual dimorphisms in feeding behaviour and a higher incidence of disordered eating in female than male populations.

### Keywords

Habitual learning; Substantia nigra; Lateral hypothalamus; Hypocretin; SB-334867; Junk food diet



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Statement of contribution

SM and TMF performed research and data analysis. TMF designed the research and wrote the manuscript. SM critically reviewed the manuscript.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## 1. Introduction

The consumption of calorie-dense foods is associated with increased mortality and obesity, and a range of diseases including diabetes, hypertension, heart disease, cancer, and kidney disease [17,30]. Despite these poor health outcomes, and the desire to reduce intake, these foods are often consumed in excess and reducing intake is recognised to be difficult [13,30,32]. One neurocognitive process that is likely to contribute to this loss of control of food intake is the development of habitual behaviour [13,32], which is also proposed to contribute to addiction to drugs of abuse [21,53]. Habits are automated behaviours that can occur with repeated practice of an action and are driven by environmental stimuli (i.e., stimulus-response associations) rather than by their associated goal or outcome (i.e., response-outcome associations) [13,21,53,64,71]. Thus, habitual actions are relatively resistant to change and persist when the value of the associated outcome has been decreased, unlike goal-directed actions which are flexible and are reduced when the associated outcome is devalued [21,53,64]. Exposure to calorie-dense foods accelerates habitual control of food seeking in rats as they will continue to press a lever for a food outcome after it has been devalued (by free-feeding it to satiety) [23,34]. In contrast, rats limited to a standard chow diet display goal-directed food-seeking behaviour, as they are sensitive to devaluation and will reduce lever press responses following outcome devaluation [23,34].

The neurocircuitry known to regulate the development of habitual behaviour centres on the dorsolateral striatum with dopamine recognised to be particularly important for accelerated habit learning associated with drugs of abuse and high-calorie diet (HCD) [21,23,27,64]. The orexins (ORX) are two lateral hypothalamic-based neuropeptides (ORX-A and ORX-B) that are recognised to be involved in appetite regulation, reward-based eating, and motivation for natural rewards and drugs of abuse [2,11], but are yet to be implicated in habitual actions. Both ORXs are known to act at two receptors, ORX-receptor-1 and −2 (ORX-R1 and ORX-R2) which are located in many brain regions [31,42], including those known to regulate habitual behaviour [21,64,71]. Specifically, ORX-R1 which is thought to have a greater role in motivated behaviours than ORX-R2 [11,42], is found in the central nucleus of the amygdala, infralimbic cortex, dorsal striatum, substantia nigra pars compacta (SNc), and on dopamine neurons in the SNc [31,38,42]; regions that when lesioned reduce habitual behavior [4,35,37,43,74]. Furthermore, ORX-R1 has also been shown to be necessary for cue-driven feeding as antagonism of ORX-R1 with SB-334867 reduces operant responding for calorie-dense foods [48,62], cue-induced sucrose seeking [12], and cue-induced reinstatement of sucrose seeking [10]. Thus, ORX-R1 might also modulate stimulus-response associations associated with habitual food-seeking.

In the current study, we investigated whether antagonism of ORX-R1 regulates habitual actions. Male rats were first given ‘binge-like’ access to a high-calorie food (sweetened condensed milk; SCM) or standard chow diet and then subsequently trained to press a lever for a food outcome [23]. After devaluation of the food outcome, rats were treated with SB-334867 or vehicle prior to testing goal-directed versus habitual lever-press response rates [23]. In study 1, SB-334867 was administered systemically, and in study 2, SB-334867 was administered into SNc, and habitual behaviour associated with HCD were reduced

with SB-334867 treatment in both studies. This study showed that ORX-R1 mediates the expression of habitual actions, and that the SNc is a site of action for this effect.

## 2. Methods

### 2.1. Subjects

64 Male Long-Evans rats were obtained from Charles River Laboratories (330–380 g; Raleigh, NC, USA). Rats were housed in pairs in a temperature and light controlled colony room (12:12 h, lights on at 6 AM). Animals had access to standard chow and water at all times, except during the operant training period when food was restricted to maintain body weight at 90% of free-feeding body weight. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Utah (#15–03013), and were in accordance with the *Guide for the Care and Use of Laboratory Animals* (8th Ed., National Research Council).

### 2.2. Diet and drug treatments, and groups

Rats were initially assigned to either the high-calorie diet (HCD) group or the control-diet group (CON group). The HCD group were given restricted (or binge-like) access to sweetened condensed milk (SCM) (Nestle, Springville, UT, USA) for two hours each day for six weeks [23]. Restricted access to SCM was utilized as we have previously shown that it promotes habitual behaviour while continuous access to SCM does not [23]. The SCM (73% sugar, 10% fat, 10% protein) was diluted to 20% in water so that the final concentration was 1 kilocalorie/g. Each day, rats were moved from the colony room to a different room, and one of two plastic water bottles was removed from the homecage and replaced with a glass bottle containing SCM. The CON group were treated in a similar manner except that the glass bottle contained water. Chow and water were available to both groups at this time (and at all times). Bottles containing SCM were weighed daily and the total consumption for each cage was divided by the number of animals per cage (2 per cage) to obtain the approximate average intake per animal each week. Every animal was observed to consume some SCM at the start of the session each day. Animals were weighed twice each week during this period, which was averaged to obtain the average body weight each week. The diet pre-treatment phase ceased prior to commencement of instrumental training (see Fig. 1).

Groups were treated with the ORX-R1 antagonist, SB-334867 (SB), or vehicle (VEH) prior to testing sensitivity to outcome devaluation (i.e., after the devaluation free-feeding period and prior to the operant-extinction test; Fig. 1). SB-334867 was obtained from Tocris Bioscience (Elllisville, MO, USA). For study 1, a low dose of SB-334867 [2,11] was administered via intraperitoneal injection 30 min prior to test (5 mg/kg in a volume of 10 mg/ml) [7,61]. The SB-334867 was dissolved to form a suspension in 10 % hydroxypropyl-beta-cyclodextrin and 2 % dimethyl sulfoxide in sterile water using a vortex and a 60 °C heat bath. The VEH solution was made and treated in the same way, but without the addition of the SB-334867. For study 2, SB-334867 was administered via intracranial cannula targeted at the SNc 5 min prior to test (4 nmol/0.4 μl; in vehicle of 1% hydroxypropyl-beta-cyclodextrin and 0.2% dimethyl sulfoxide in sterile water). This is a relatively low

dose of SB-334867 which has been shown to alter cue-driven drug-seeking behaviour when administered into the ventral tegmental area, immediately adjacent to the SNc [29,41]. Hence, the groups for both study 1 and study 2 were CON-VEH ($n$=8), CON-SB ($n$=8), HCD-VEH ($n$=8), HCD-SB ($n$=8) indicating diet-drug treatments in each case.

### 2.3. Intracranial implantation, infusions, and histology

Across the 1-week period after the diet-treatment phase ceased (Fig. 1), animals in study 2 were implanted with intracranial cannula targeted at the lateral part of the SNc which has been shown to provide dopaminergic input to the dorsolateral striatum [4]. Surgery was conducted in a stereotaxic frame (Stoelting Co, IL, USA) under isoflurane anaesthesia (5% induction, 2% maintenance) combined with analgesics (buprenorphine 0.06 mg/kg, i.p., and tetracaine hydrochloride, 0.25 mg, s.c.) and penicillin (300 units/kg, i.m.). Burr holes were made in the skull and bilateral guide cannula (26 gauge with dust caps; Plastics One, VA, USA) implanted 1 mm dorsal to the SNc (−5.2 mm posterior, ±2.2 mm lateral and −7.0 mm ventral to bregma; [58]. Cannula were fixed to the skull using stainless steel screws and acrylic dental cement. Post-surgery, animals were monitored daily for 7 days prior to commencement of operant training.

Bilateral infusions of SB-334867 or VEH were made into the SNc via infusion cannula (33 gauge; Plastics One) which projected 1 mm ventral to the guide cannula (making the final target −8.0 mm ventral to the skull). Infusion cannulas were connected via polyethylene-50 tubing to 25 μl Hamilton syringes which were mounted on an infusion pump. Infusions (0.4 μl per side) were made over 2 min and the cannula left in place for a further 1 min to allow for diffusion of the drug.

Cannula placements were made on completion of behavioural experiments, whereby rats were overdosed with sodium pentobarbital (140 mg/kg), the brains removed, and flash frozen in isopentane. Brains were then sectioned on a cryostat (40 $\mu$m), mounted onto glass slides, Nissl stained, and cannula placements plotted on templates from [58].

### 2.4. Operant training

Operant training took place in 12 Coulbourn chambers with Plexiglas and stainless-steel walls and a wire floor over a tray of paper bedding [30 cm (width) × 25 cm (length) × 29 cm (height); Coulbourn Instruments, Whitehall, PA]. On one wall of each chamber there was a retractable lever located to the left-side of a recessed trough. The chambers were illuminated with an individual 3-W house-light located on the opposite wall, and were contained within sound-attenuating cabinets equipped with ventilation fans. When appropriate, lever pressing resulted in the delivery of a 45 mg pellet into the trough from a pellet dispenser. For half of the animals, the pellet was grain-based (Bio-Serv, NJ, USA) and for the other half of the animals the pellet was sugar-based (TestDiet, MO, USA). The pellet that was not used at training was utilized as the non-devalued condition at test (as described in Section 2.5), with performance at training and test combined for the two counter-balanced conditions [22,25,27,63]. Pellet types were calorically similar (3.35 kcal/g and 3.4 kcal/g for grain-based and sugar-based, respectively). The timing and recording of all events were controlled by Graphic State 3.0 software (Coulbourn Instruments, Whitehall, PA).

Operant training took place 2 days after the end of the diet-pre-treatment phase in study 1, and 14 days after in study 2 (i.e., after intracranial implantation and recovery from surgery) (Fig. 1). Rats were initially trained to press the lever using a continuous reinforcement schedule (CRF) where each lever press resulted in the delivery of a pellet. Once 60 pellets were earned within a single CRF session, training then continued with one session each day where 60 pellets were earned within 90 min under a random interval (RI) schedule. The RI increased each day as follows: one session of RI-15, one session of RI-30 and four sessions of RI-60 where an average interval of 15, 30 and 60 s occurred before lever press resulted in pellet delivery [23,26,27,63].

### 2.5. Outcome devaluation testing

Animals were tested for goal-directed responding using an outcome-devaluation procedure [23,53]. Specifically, each animal was allowed to freely consume one type of pellet in a transparent plastic tub for one hour so that the pellet would be devalued by sensory-specific satiety. The rats were then tested for 5 min in the operant chamber where lever press was recorded but no pellets were delivered (i.e., extinction conditions). Following the free-feeding devaluation period (and before the operant-extinction test), rats in study 1 received an i.p. injection of either vehicle or SB-334867 and were placed in a clean plastic tub for a further 30 min without any pellets available, and rats in study 2 were infused with vehicle or SB-334867 after free-feeding devaluation period and placed in a clean plastic tub for 5 min, prior to testing in the operant chamber. Each rat was tested twice under devalued and non-devalued conditions two days apart with one re-training session of RI60 in between. For each test, rats were infused with SB-334867 or vehicle, i.e., each rat was either treated twice with SB-334867 or twice with vehicle. The amount of food consumed during the free-feeding period was recorded and averaged across the two sessions. Lever response rates at test were corrected for each rat by dividing it by the rate of responding during the final day of training [27,27,35,37,43,66,74].

Following operant tests, rats in study 1 were placed directly back in clean plastic tubs for 10 min with access to a pellet outcome that was either the same (i.e. devalued) or different (i.e. non-devalued) to the pellet that was consumed prior to test, in a counterbalanced manner [23,24]. This consumption test was used to confirm that the pre-fed pellet was appropriately devalued (i.e., that sensory specific satiety was intact), and to examine consumption of the pellets under SB-334867 compared to vehicle [1,23,55,57].

### 2.6. Statistical analysis

Body weight, operant training and outcome devaluation test data were analysed as mixed 2 × 2 × 2 ANOVAs (between subjects factors were diet and drug to be administered, and within subjects factor week, training session or devaluation condition, respectively). Pre-test free-feeding data was analysed as a 2 × 2 ANOVA (diet and drug to be administered). Statistical significance was set at $p < 0.05$ for all analyses. When appropriate simple effect analyses were conducted to determine the source of any significant interactions. Data are presented as mean ± SEM.

# 3. Results

## 3.1. Study 1: systemically administered ORX-R1 antagonist

In study 1, one rat did not learn to press the lever within six CRF sessions and was removed from the study making group numbers as follows: CON-VEH ($n$=7), CON-SB ($n$=8), HCD-VEH ($n$=8), HCD-SB ($n$=8).

**3.1.1. Caloric intake and body weight—**The average daily kilocalories of SCM consumed each day for the 6-week diet period for rats in the HCD group was 17 (±1), 21 (±1), 25 (±2), 25 (±2), 29 (±2) and 28 (±2) kcal [average (±SEM); similar to prior report [23]]. There were no differences in body weight for animals exposed to HCD versus control diet [see Fig. 2A; consistent with [23]; main effect of week ($F_{(1,29)}$=686.8, $p$<0.0001) and no differences between groups or interactions ($F_{(1,29)}$<0.5, $p$>0.4, for all analyses)].

**3.1.2. Operant training—**Preliminary analysis showed that there was no differences in response rates for grain versus sucrose pellet at training ($F_{(1,27)}$=2.08, $p$=0.16), so data was collapsed across this variable into 4 groups. Rates of lever pressing increased across instrumental training and did not differ between the four groups (Fig. 2B). Statistical analysis confirmed a main effect of training session ($F_{(1,27)}$=105.0, $p$<0.001), and no effect of diet ($F_{(1,27)}$=2.7, $p$>0.1), drug to be administered ($F_{(1,27)}$=0.1, $p$=0.78) or any interactions ($F_{(1,27)}$<0.5, $p$>0.5, for all analyses).

**3.1.3. Outcome devaluation tests—**There was no difference between groups in the average amount of pellets consumed in the free-feeding period prior to the operant tests [Fig. 2C; no main effect of diet ($F_{(1,27)}$=0.02, $p$>0.9), drug to be administered ($F_{(1,27)}$=2.5, $p$>0.1), or interaction ($F_{(1,27)}$=1.5, $p$>0.2)]. The results of the operant test are shown in Fig. 2D. Preliminary analyses included pellet type as a factor and since there was no interaction between pellet type and any other factor ($F_{(1,23)}$<0.61, $p$>0.44, for all analyses), data was collapsed across this variable to make 4 groups. There was also no impact of test order on these factors ($F_{(1,27)}$<1.2, $p$>0.44, for all analyses) and no differences in total response rates between groups prior to correcting the raw data by baseline ($F_{(1,27)}$<3.2, $p$>0.09, for all analyses). Control animals (CON-VEH group) were goal-directed in their behaviour, reducing lever response rates under devalued conditions compared to non-devalued conditions. In contrast, as expected HCD vehicle-treated animals were habitual (HCD-VEH group) pressing the lever equally under devalued and non-devalued conditions. Importantly, SB-334867 administration rescued this effect of HCD as animals remained goal-directed (HCD-SB group). Statistically, there was no main effect of diet ($F_{(1,27)}$=0.4, $p$>0.5), but a main effect of drug treatment ($F_{(1,27)}$=5.2, $p$=0.03) and no interaction ($F_{(1,27)}$=1.1, $p$>0.3) suggesting that SB-334867 reduced lever-responses overall. There was also a main effect of devaluation condition ($F_{(1,27)}$=10.0, $p$<0.01) and no interaction between devaluation condition and diet, or drug ($F_{(1,27)}$<0.3, $p$>0.5, for all analyses). Importantly, there was a 3-way interaction between diet, drug and devaluation ($F_{(1,27)}$=5.8, $p$=0.02). Analysis of simple effects to examine the source of this interaction revealed that while CON-VEH and HCD-SB groups demonstrated sensitivity to outcome devaluation ($p$<0.05), the CON-SB and HCD-VEH groups did not, responding equally under devalued and

non-devalued conditions ($p$>0.05). If lever response rates were simply suppressed when insensitivity to outcome devaluation was demonstrated, it would be problematic to an interpretation that habitual actions had occurred. Thus, simple effects analysis were also carried out to compare responding by the CON-SB and HCD-VEH groups to the control group (CON-VEH group). Analysis revealed that there were no differences in lever response rates under devalued conditions ($p$=0.43) or under non devalued conditions ($p$=0.22) for the HCD-VEH group, but lever response rates were reduced for the CON-SB under non-devalued conditions ($p$=0.003).

Finally, for post-test consumption, all groups decreased consumption when the devalued outcome was freely available compared to the alternate, non-devalued outcome (Fig. 2E). This was confirmed by a main effect of devaluation ($F_{(1,27)}$=35.2, $p$<0.001), which did not interact with diet or drug ($F_{(1,27)}$<1.8, $p$>0.1). There was also no main effect of diet ($F_{(1,27)}$=1.1, $p$>0.3), but there was a main effect of drug ($F_{(1,27)}$=13.3, $p$<0.001) which occurred because the SB groups consumed less than the VEH groups overall.

### 3.2. Study 2: intra-nigral ORX-R1 antagonist

In study 2, a total of 7 rats were removed from all analysis due to cannula injection sites outside of the SNc, making final group numbers CON-VEH ($n$=6), CON-SB ($n$= 7), HCD-VEH ($n$= 6), HCD-SB ($n$= 6). Injection sites for animals included in the analysis are shown within the SNc in Fig. 3A.

**3.2.1. Caloric intake and body weight—**The average daily kilocalories of SCM consumed each day for the 6-week diet period for the rats in the HCD group was 14(1), 19 (1), 25 (1), 25 (1), 26 (1) and 26 (1) kcal [average (±SEM)]. There were no differences in body weight for animals exposed to HCD versus control diet [Fig. 3B; main effect of week ($F_{(1,23)}$=843.2, p<0.0001) and no effect of groups or interactions ($F_{(1,24)}$<0.07, $p$>0.790), for all analyses)].

**3.2.2. Operant training—**Data from the first 3 days of training was lost due to computer failure. Data from the final 4 days of training is shown in Fig. 3C. Preliminary analysis showed no differences in response rates for grain pellet versus sucrose pellet at training ($F_{(1,27)}$=0.02, $p$=0.89), so data was collapsed across this variable into 4 groups. There were no differences in response rates between the four groups (no effect of training session, diet, drug to be administered, or any interactions; $F_{(1,27)}$<3.09, $p$>0.09, for all analyses) as seen in study 1 and [23].

**3.2.3. Outcome devaluation tests—**There was no difference between groups in the average amount of pellets consumed in the free-feeding prior to the operant tests (Fig. 3D; no effect of diet, drug to be administered, or interaction; $F_{(1,21)}$<1.3, $p$>0.26, for all analyses). The results of the operant test are shown in Fig. 3E. Preliminary analyses included pellet type as a factor and since there was no interaction between pellet type and any other factor ($F_{(1,17)}$<0.79, $p$>0.41, for all analyses), data was collapsed across this variable to make 4 groups. There was also no impact of test order on these factors ($F_{(1,23)}$<2.1, $p$>0.17, for all analyses) and no differences in total response rates between groups prior to correcting

the raw data by baseline ($F_{(1,27)}$<0.27, $p$>0.61, for all analyses). As expected, control animals were goal-directed in their behaviour (CON-VEH group) whilst HCD-vehicle treated animals were not (HCD-VEH group). SB-334867 restored goal directed behaviour in HCD-exposed rats (HCD-SB group). Statistically, there was no main effect of diet ($F_{(1,23)}$=1.1, $p$=0.31) or drug treatment ($F_{(1,23)}$=0.01, $p$=0.92) and no interaction ($F_{(1,23)}$=2.2, $p$=0.15). There was also a main effect of devaluation condition ($F_{(1,23)}$=15.6, $p$<001) and no interaction between devaluation condition and diet, or drug ($F_{(1,23)}$<2.1, $p$>0.16, for all analyses). Importantly, there was a 3-way interaction between diet, drug and devaluation ($F_{(1,23)}$=4.7, $p$=0.04). Analysis of simple effects to examine the source of this interaction revealed that there was no difference between devalued and non-devalued conditions for the HCD-VEH ($p$>0.05), but there was a significant difference for the other three groups ($p$<0.05). Finally, when lever response rates of the HCD-VEH group were compared to control (CON-VEH group), there were no differences under devalued conditions ($p$=0.29) or under non devalued conditions ($p$=0.20). Post-test consumption was not examined in study 2.

## 4. Discussion

The current study demonstrated that antagonism of ORX-R1 (using SB-334867) reduces habitual food-seeking of rats exposed to high-calorie diet (HCD). Specifically, after restricted ‘binge-like’ access to sweetened condensed milk (SCM), rats were trained to press a lever for a food outcome and were then tested for goal-directed versus habitual behaviour using a satiety devaluation procedure. At test, rats exposed to HCD demonstrated equivalent lever response rates under devalued conditions compared to non-devalued conditions, and hence were habitual in their actions, whilst rats exposed only to chow reduced lever responses under devalued conditions and were hence goal-directed in their actions, as we have previously shown [23]. Importantly, treatment with SB-334867 prior to test restored goal-directed behaviour in the HCD-exposed animals, when administered systemically (study 1), as well as directly into SNc (study 2). Hence, this study shows for the first time that ORX-R1 has a role in modulating the expression of habitual actions, and that an important locus for this effect is the SNc. Thus, this study extends on the neurocircuitry known to regulate habitual actions to include the ORX system, and also suggests that targeting ORX-R1 is a potential way to regain behavioural control of food intake.

The neurocircuitry known to regulate habitual actions centres on the dorsolateral striatum and is distinct from the neurocircuitry regulating goal-directed actions which centres on the dorsomedial striatum [21,64]. ORX-R1 are expressed in several brain regions that are necessary for the expression of habitual actions, including dorsolateral striatum, central nucleus of the amygdala, infralimbic cortex, and SNc [31,42], and thus SB-334867 could have acted at any of these locations when administered systemically to reduce habitual behaviour in the current study. We confirmed that the SNc is an important locus, and further studies would be required to determine whether ORX-R1 also acts at other brain regions to regulate habits. Given that SB-334867 is an ORX-R1 antagonist it may have acted to offset an increase in released ORX peptide, or an increase in expression or sensitivity of ORX-R1, that results from exposure to HCD. Indeed, the consumption of calorie-dense food has been shown to increase the number of hypothalamic ORX-expressing neurons

and ORX mRNA [52,69,72], which may in turn increase ORX activity in the SNc, and possibly other ORX-terminal regions, to promote habits. Notably, a similar mechanism has been proposed to account for some of the drug addiction-promoting effects of ORX [18] as exposure to many drugs of abuse increases the number of ORX-expressing neurons [18,54,68], and SB-334867 reduces drug-seeking behaviours [18,19,46,47]. Interestingly, intermittent drug exposure, that promotes stronger drug-seeking behaviours than longer continuous drug exposure, leads to a greater increase in ORX-expressing neurons, and lower doses of SB-334867 are required to alter these behaviours than following continuous drug access [18]. This study, as well as other studies, demonstrate that the greater the impact on the ORX system, the stronger the addiction-like behaviours, and the more sensitive the behaviour to SB-334867, which likely reflects a critical role of ORX-R1 signalling in maintaining these behaviours [18,19,46,47,54]. This is not unlike the idea that palatable food and binge-like consumption may be more dependent on ORX-R1 signalling than standard diets, as lower doses of SB-334867 are also often required to reduce these behaviours [2,45]. Thus, it may be that the low dose of SB-334867 that was used in the current study (5 mg versus 10–30 mg/kg; [2]) was effective at restoring goal-directed behaviour because the HCD-potentiated habits were particularly under the control of the ORX system.

Within the SNc, most neurons produce dopamine (85% of total neurons) and project to the striatum as well as to other brain regions including the cortex, subthalamus, globus pallidus, and amygdala [16,73]. ORX terminal fibres within the SNc are in close apposition to dopamine neurons across its medial-lateral extent [5], and nigral dopamine neurons are highly co-localised with ORX-R1-expressing neurons (i.e., near 100% co-localised) [39], which suggests that SB-334867 acts directly on dopamine neurons in the SNc. The relationship between ORX and non-dopamine neurons in SNc has yet to be systematically investigated but a small number of non-dopamine neurons may also express ORX-R1 (see Fig. 2 of [38]). In any case, it has been demonstrated that SB-334867 reduces basal firing rate of nigral dopamine neuron in vitro, as well as in vivo following increased activity that results from chronic haloperidol administration (Rasmussen, Hsu et al. 2007, [38]), thus indicating that ORX-R1 antagonism reduces activity of dopamine neurons. Dopamine is recognised to play an important role in the development of habitual actions as excess dopamine has been shown to accelerate habits [21,24,27,49], and dopamine deficiency prevents habits [4,43]. In particular, dopamine in the dorsolateral striatum is important as D1-anatagonists administered into dorsolateral striatum prevents the transition to habits following exposure to HCD [23]. The lateral aspects of the SNc that were targeted in the current study preferentially provide dopamine input to the dorsolateral striatum [4,16], and thus one possibility is that SB-334867 administered to the SNc decreased dopamine in the dorsolateral striatum to prevent habits.

Habits are generally adaptive behaviours that allow for efficient, automated interaction with the environment when an action is well-practiced [71]. However, habitual actions can also be problematic when associated with a loss of behavioural control as occurs following exposure to drugs of abuse or HCD [21,23,24,27,49,53]. The current study adds to the growing literature which demonstrates that antagonism of ORX-R1 reduces stimulus-driven eating behaviour, as well as stimulus-driven drug-seeking behaviour ([10,11], Cole, Mayer et al. 2015, [18,28]). Therefore, targeting ORX-R1 may have therapeutic potential to regain

control of food intake in compulsive over-eating, binge eating disorder and obesity [11,33], as has been proposed for drug addiction [11,28]. Such treatment, which targets behavioural modification, would be effective in conjunction with current treatments which tend to target energy regulatory systems and appetite [65]. Importantly, ORX-R1 alters both habitual behaviour and appetite as the SB-334867 treated groups reduced pellet intake during the post-test free-feeding period and reduced lever response rates overall at test (compared to VEH groups). This is consistent with prior findings using SB-334867, and suggests that ORX-R1 antagonism reduces motivation for food and willingness to work for food [2,11,62]. Reduced motivation for food is also likely to account for the effect of SB-334867 on control-diet animals at test. That is, a floor effect resulting from significantly suppressed responding is likely to underlie the equivalent lever responses at test under devalued and non-devalued conditions by the CON-SB group (rather than deficits in goal-directed behaviour, described in more detail below). The reason why this floor effect did not occur for the HCD-SB group may be because the impact of SB-334867 on motivated responding was lessened due to neuroadaptations resulting from the HCD (such as increased ORX or ORX-R1, as discussed above). Thus, for the CON-SB group there would be less basal ORX activity or ORX-R1 than the HCD-SB group, and therefore a greater impact of SB-334867 on ORX-R1 and subsequently on motivated responding for the CON-SB group than for the HCD-SB group. In any case, the reduced lever-response rates of the CON-SB at test were only seen when SB-334867 was systemically administered and not when it was administered directly into the SNc. ORX is thought to orchestrate various components of appetitive behaviour across different neurocircuits and is known to have selective functions at its different terminal regions [2,40,51]. The current study demonstrates that motivation for food (as indicated by lever response rates) occurs outside of the SNc, in a circuit independent of that which regulates habitual actions. Prior studies show that SB-334867 administered into the nucleus accumbens or ventral tegmental area reduces food intake [44,67,75], making the ventral, rather than dorsal, striatal circuit a possible site for these effects when SB-334867 was systemically administered in the current study [3].

The current study used binge-like exposure to HCD where large amounts of calories were consumed by rats within a restricted period of time [14]. This schedule was chosen as we have previously demonstrated that it promotes habitual actions, unlike continuous exposure to SCM where rats remain goal-directed in their behaviour as with standard chow-fed animals [23]. Other studies have similarly shown that habitual behaviour develops following restricted periods of calorie intake in rats, including intermittent access to a sucrose solution [34], and after repeated cycles of food restriction and refeeding of standard chow [56]. Such binge-like consumption of food in rats is thought to model binge eating disorder, which is characterised by repeated episodes of excessive, non-homeostatic consumption of food [2,14,45]. Importantly, the ORX system is recognised to be dysregulated in binge eating disorder, and targeting the ORX system has recently been suggested as a possible target for pharmacotherapy [2,45] in line with the current findings. Of note, however, there are recognised sexual dimorphisms in feeding behaviour, and disordered eating is far more prevalent in female than male populations [50], hence it would be imperative to establish whether targeting ORX-R1 is effective at reducing habitual actions in female rats given that only males rats were examined in the current study. Importantly, a similar reduction

in consumption and motivation for palatable food has been reported in female rats as male rats with SB-334867 [9,20], and a similar reduction in habitual food seeking in female rats would greatly strengthen the idea of ORX-R1 antagonists as a multi-faceted approach to treat disordered eating that targets total food consumption as well as learned behaviours associated with feeding.

Finally, it is worth considering whether the lack of sensitivity to outcome devaluation at test by the HCD diet could have occurred for reasons other than habitual behaviour. One potential confound is suppressed lever response rates which would be difficult to reconcile with habitual behaviour and may occur, for example, because of some impact of HCD on motivation or capacity to work for reward [66](as described for the CON-SB group above). However, the HCD-VEH group did not demonstrate a significant reduction in response rates at test, and thus were not more sensitive to any potential non-specific, response-suppressing effects of the devaluation procedure than control rats (CON-VEH group) [36]. Nonetheless, many prior studies show that habitual animals tend to respond less under non-devalued conditions than animals that are goal-directed ([23–24, 34,35,74], Parkes, [4,24,27,36,63]), but most often these reductions are also not likely to have reached statistical significance. Furthermore, across these studies, habits also tend to be associated with increased responding under devalued conditions, which is not easily accounted for by a general reduction in motivation. Instead, one possibility is that the satiety procedure used for devaluation differentially influences response rates for goal-directed versus habitual actions. For goal-directed actions, there is a representation of the outcome that drives responding [15,53], and hence animals can act selectively to achieve that outcome, and satiation would reduce responding at test only when the lever is associated with the sated outcome. In contrast, for habitual actions the outcome is not represented and does not drive responding, which are instead driven by Pavlovian-associated environmental stimuli and thus by a general rather than specific appetitive drive [53,59,70,71]. Thus, the animal likely responds in a sated manner (equally) under both non-devalued and devalued conditions. This generalisation would serve to reduce responding overall compared to when responding occurs for a specific goal that is not sated, but it would be higher than when that goal is known to be sated (i.e., the devalued conditioned). Thus, responses rates for habitual animals would fall somewhere between those of goal-directed animals under devalued and non-devalued conditions, and in the current study these differences were not significant, indicating that any such impact was at best minor and did not interfere with the expression of habitual actions.

It should also be considered whether the observed insensitivity to devaluation at test occurred instead because of a deficit in goal-directed actions. Such deficits could occur if the response-outcome associations had not yet formed, for example when instrumental training using RI-schedules is limited [25,70]. However, this possibility is unlikely given that goal-directed behaviour is seen following exposure to SCM with less training than that used in the current study, and only with further training, equivalent to the current study, that insensitivity to outcome devaluation emerges [63]. The most parsimonious explanation for why goal-directed behaviour was lost in this instance would be that habitual behaviour developed with extra training [25,53,71]. Other studies similarly confirm that HCD-exposed rats are not slower to develop goal-directed behaviour than control animals, and that with

further training habitual behaviour subsequently develops [34,66]. Importantly, given that these studies also used satiety to test for sensitivity to devaluation they additionally show that habitual actions can be demonstrated using satiety [34,63,66], as this procedure has been recently shown to be less effective for demonstrating habits than when lithium chloride taste aversion is used for devaluation [8,70]. Finally, it is worth noting that it has been demonstrated that HCD can prevent the expression of goal-directed behaviour [36]. In this case, the diet treatment occurred after instrumental training and therefore did not have opportunity to influence the development of habits [36] unlike when the diet treatment occurs prior to instrumental training [23,34,66]. One possible reason for this deficit is that a diverse 'cafeteria-style' diet was used, which has been shown to interfere with sensory specific satiety processes [60], which would in turn prevent goal-directed actions as they depend on the capacity for evaluating food appropriately [23,53,55,57]. In contrast, when exposed to a consistent HCD diet of either SCM, lard or sucrose, sensory specific satiety remains intact (Fig. 2E) as intake of a recently consumed (devalued) food is reduced compared to a distinct (non-devalued) food [23,34,66]. Thus, it may be that differences in the types or variety of high-calorie foods, or the access schedule of those foods [1], determine whether the animals remain sensitive to the sensory aspects of food and thus whether goal-directed behaviour will be expressed [23,55,57].

In conclusion, the current study demonstrated that habitual food seeking in rats is reduced by antagonism of ORX-R1 following exposure to high-calorie diet. This finding adds to growing evidence that targeting ORX may be a potential treatment for over-eating, and highlights that ORX-R1 antagonism can modify behaviour in addition to altering appetite which is likely to be key to treating disordered eating [2,65]. Further work is required to determine the mechanism for the effects of SB-334867, i.e., whether it acts to rectify neuroadaptations to high-calorie diet or not, and whether ORX mediates habitual actions that occur by other means, such as following exposure to drugs of abuse or during over-training of well-learned actions [21]. Given that ORX is produced exclusively by neurons within the lateral hypothalamus (LH) [6], our findings suggest that the LH may be part of the habit neurocircuitry, which has not been previously considered, and that the LH provides input to the habit neurocircuitry via the SNc. In turn, there are several other 'feeding' neuropeptides produced by the LH that may also regulate habits, such as melanin-concentrating hormone, galanin, and neurotensin [6], which could be investigated in the future to greatly extend the existing opportunities for modulating and treating habitual behaviour. Overall, this study contributes to the known function of ORX which is to translate motivation into action [40]. That is, ORX is thought to promote goal-driven, motivated behaviours to acquire both homeostatic and non-homeostatic rewards [2,3,40]. This study highlights the important role that environmental stimuli and learned associations (i.e., stimulus-response associations) can play in driving these actions [11], and in turn provides a basis for how maladaptive behaviours may emerge following exposure to highly potent rewards and persist despite the desire for change [13,21,32].

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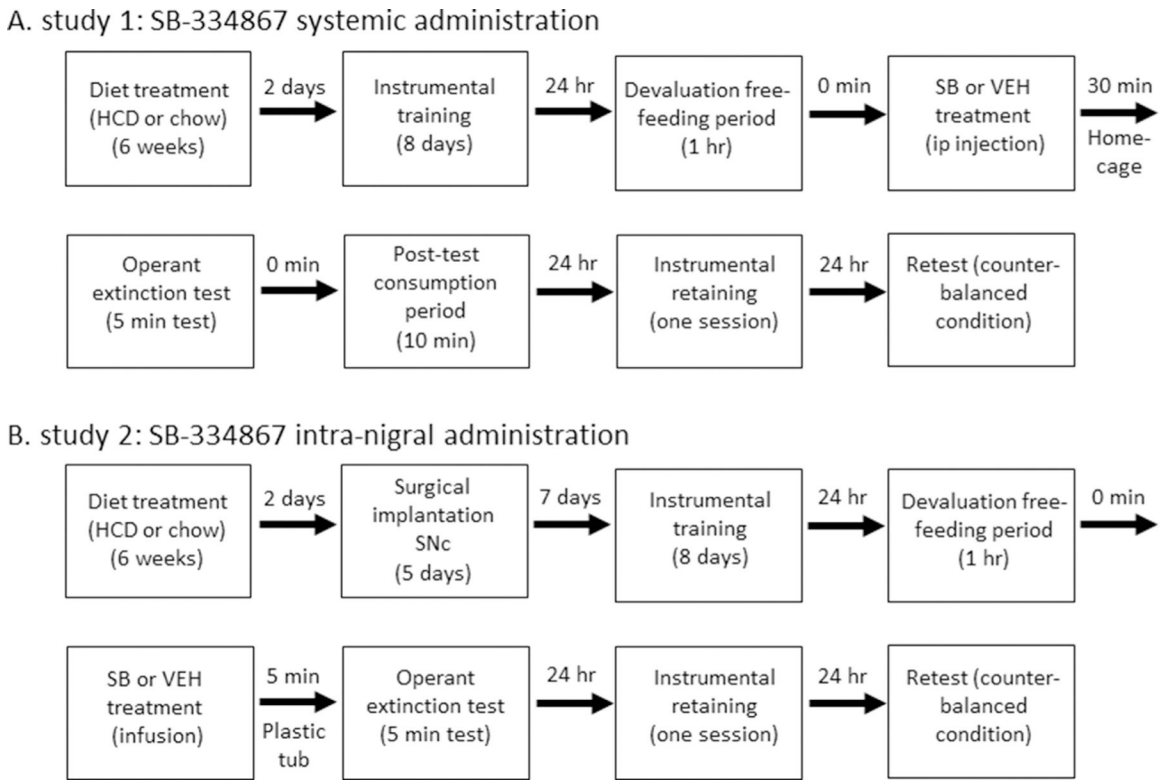


**Fig. 1.**

Timeline of experimental procedures. A. Systemic administration of SB-334867. Rats were given 6-weeks exposure to high calorie diet (HCD) or standard chow. Rats were then trained to press a lever for a pellet outcome before testing for habitual behaviour using a satiety devaluation procedure where the pellet was freely available for 1 hour. Immediately after the devaluation period, rats were administered an intraperitoneal injection of either SB-334867 or vehicle and then lever response rates tested in extinction (30 min later). Immediately following test, the same or different pellet was freely available to examine the effectiveness of the devaluation procedure and the impact of SB-334867 on consumption. Rats were then re-tested in extinction, after an instrumental retraining session, where the other pellet outcome was devalued (and the same drug was administered as in the first test). B. Intra-nigral administration of SB-334867. Following the 6-week diet phase, rats were surgically implanted with intracranial cannula targeted at the substantia nigra compacta (SNc). One week after recovery from surgery, rats were trained and tested for habits using a satiety devaluation procedure (as in study 1). Either SB-334867 or vehicle was infused into SNc after the devaluation period but prior to testing lever response rates. Rats were tested twice under devalued and non-devalued conditions where the same drug was infused into SNc for each test.

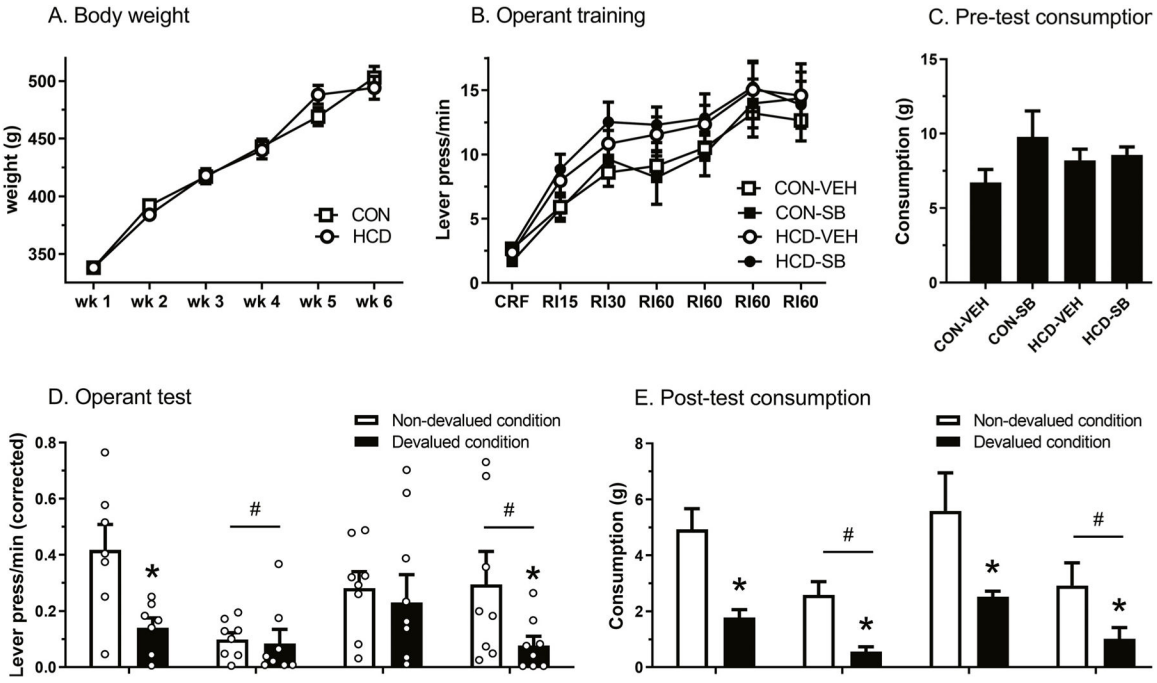


**Fig. 2.**

Systemic administration of SB-334867. A. Body weight. Average body weight across the 6-week diet period did not differ for the groups exposed to high calorie diet (HCD) compared to control diet (CON). Note that the SEM error bars are smaller than the symbols in some instances. B. Operant training. All groups learned to press a lever for a pellet outcome using a random-interval (RI) training schedule with no differences between groups in lever response rates. C. Pre-test consumption. There was no difference between groups in the amount of pellets consumed during the free-feeding devaluation period prior to operant testing. D. Operant test. Control animals (CON-VEH group) demonstrated goal-directed behaviour at test reducing responding on the lever under devalued conditions compared to non-devalued conditions. In contrast, animals exposed to a high-calorie diet (HCD-VEH group) pressed the lever equally under devalued versus non-devalued conditions and therefore displayed habitual behaviour. Importantly, SB-334867 treatment restored goal-directed behaviour in animals exposed to high-calorie diet (HCD-SB group). SB-334867 reduced lever response rates overall, which may have resulted in a floor effect for the control-diet rats treated with SB-334867 (CON-SB group). Individual performance of each rat is show as a white circle. E. Post-test consumption. All groups displayed reduced consumption of the devalued outcome compared to the non-devalued outcome when it was freely available and thus demonstrated intact sensory specific satiety. SB-334867 reduced the amount of food consumed overall compared to vehicle (VEH), suggesting that it reduces motivation for food. ∗ Indicates significant simple effect of devaluation condition. # Indicates significant main effect of drug treatment.

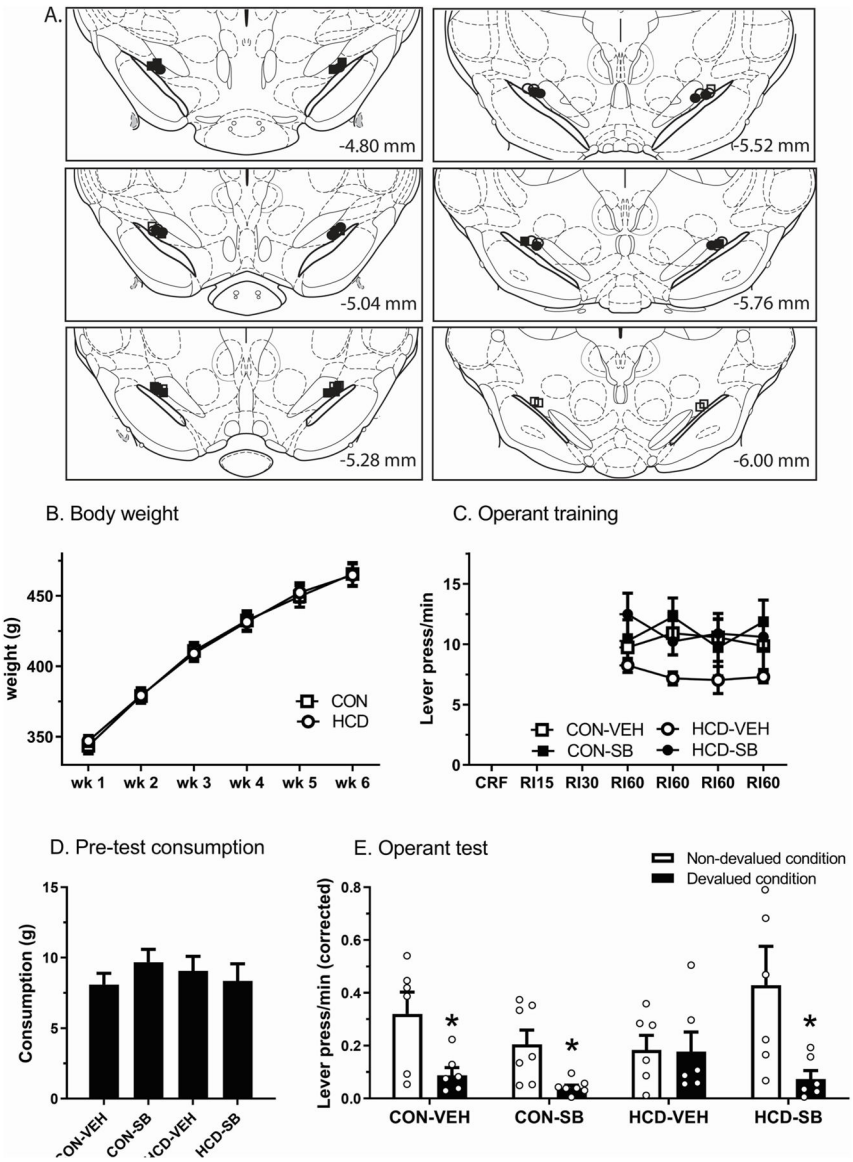


**Fig. 3.**

Intra-nigral administration of SB-334867. A. Cannula placements. Plots of cannula placements that successfully targeting the SNc of rats included in data analysis: CON-VEH group, white squares ($n$=6), CON-SB group, black squares ($n$=7), HCD-VEH group, white circles ($n$=6) and CON-VEH group, black circles ($n$=6). 7 rats were excluded from all analysis due to cannula placement outside of the SNc. Brain templates modified from Paxinos and Waston, 2006. B. Body weight. Average body weight across the 6-week diet period did not differ for the groups exposed to high calorie diet (HCD) compared to control diet (CON). C. Operant training. The first 3 days of training data were lost due to computer failure. There were no differences between groups in lever response rates across the last four random-interval (RI)-60 training sessions. D. Pre-test consumption. There was no difference between groups in the amount of pellets consumed during the free-feeding devaluation period prior to operant testing. E. Operant test. Control animals

(CON-VEH group) demonstrated goal-directed behaviour reducing lever responses under devalued compared to non-devalued conditions, whilst animals exposed to high-calorie diet demonstrated habitual behaviour (HCD-VEH group). Importantly, SB-334867 treatment restored goal-directed behaviour in animals exposed to high-calorie diet (HCD-SB group). Individual performance of each rat is show as a white circle. ∗ Indicates significant simple effects of devaluation condition.